

Animal models in type 2 diabetes research: An overview

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Type 2 diabetes is a complex and heterogeneous disorder presently affecting more than 100 million people worldwide and causing serious socio-economic problems. Appropriate experimental models are essential tools for understanding the pathogenesis, complications, and genetic or environmental influences that increase the risks of type 2 diabetes and testing of various therapeutic agents. The animal models of type 2 diabetes can be obtained either spontaneously or induced by chemicals or dietary or surgical manipulations and/or by combination thereof. In recent years, large number of new genetically modified animal models including transgenic, generalized knock-out and tissue-specific knockout mice have been engineered for the study of diabetes. This review gives an overview on the animal models of type 2 diabetes with reference to their origin/source, characteristic features, underlying causes/mechanism(s), advantages and disadvantages to the investigators in diabetes research. In addition, it especially describes the appropriate selection and usefulness of different animal models in preclinical testing of various new chemical entities (NCEs) for the treatment of type 2 diabetes.

Key words Animal models - new chemical entities - preclinical testing - type 2 diabetes

The pathogenesis of type 2 diabetes is complex involving progressive development of insulin resistance in liver and peripheral tissues accompanied by a defective insulin secretion from pancreatic beta cells leading to overt hyperglycaemia (an abnormally high amount of glucose levels in blood)¹. Despite genetic predisposition, the risk of developing type 2 diabetes in humans increases with age, obesity, cardiovascular disease (hypertension, dyslipidaemia) and a lack of physical activity^{1,2}. Generally, current therapeutic strategies for type 2 diabetes are limited

and involve insulin and four main classes of oral antidiabetic agents that stimulate pancreatic insulin secretion (sulphonylureas and rapid-acting secretagogues/insulinotropics *e.g.*, glibenclamide, glipizide, rapaglinide), reduce hepatic glucose production (biguanides *e.g.*, metformin), delay digestion and absorption of intestinal carbohydrate (α -glucosidase inhibitors *e.g.*, acarbose) or improve insulin action [thiazolidinediones (TZDs) *e.g.*, pioglitazone, rosiglitazone]. Each of above agents suffers from generally inadequate efficacy and

number of serious adverse effects. Thus, there are wide variety of newer therapeutic agents/strategies being examined for the treatment of type 2 diabetes, most of all currently under preclinical and early clinical stages of drug development^{2,3}.

Due to complex interaction among multiple susceptibility genes and between genetic and environmental factors, genetic analysis of diabetes is difficult and poorly understood in humans. Moreover, diabetes research in humans is impeded by obvious ethical considerations, because provocation of disease is strictly impermissible in man. Animal models of diabetes are therefore greatly useful and advantageous in biomedical studies because they offer promise of new insights into human diabetes. Inbred animal models, in which the genetic background is homogeneous and environmental factors can be controlled, are therefore valuable in genetic dissection of multifactorial diseases. Most of the available models are based on rodents because of their small size, short generation interval, easy availability and economic considerations; however, nonrodent models of diabetes are urgently needed as a valuable supplement to rodents for both practical and physiological reasons with respect to humans. The large number of different models developed for different traits and insufficient characterization of some models make it difficult to choose the right model for a given study (including pharmacological screening) and at times leading to misinterpretation of data or even to the wrong conclusions. Though there are some reviews^{4,5}, book chapters^{6,7} and books⁸⁻¹⁰ available on the animal models of diabetes mellitus, the aim of this review, however, is to give an apparent overview on the currently available animal models of type 2 diabetes with respect to their origin/source, characteristic features, aetiopathogenesis, advantages, and disadvantages in the study of human type 2 diabetes. Further, it especially deals with the appropriate selection and usefulness of different animal models in testing various classes of new chemical entities (NCEs) and other therapeutic modalities for the treatment of type 2 diabetes.

Animal models of type 2 diabetes and their classification

Animals exhibiting a syndrome of insulin resistance and type 2 diabetes, with characteristics similar to humans, comprise a wide range of species with genetic, experimental or nutritional causation. Some animals with inherent diabetes have pancreas with 'sturdy' beta cells capable of maintaining robust, life long insulin secreting capacity characterized by severe hyperinsulinaemia with only mild to moderate hyperglycaemia throughout the life *e.g.*, Zucker fatty rats (ZFR), *ob/ob* (obese), KK mouse and (corpulent) cp rat group. At the other end of spectrum, some species possess 'brittle or labile' pancreatic beta cells allowing only for transient insulin hypersecretion with short-term obesity. Subsequently, as a result of genetic predisposition and affluent nutrition/other environmental causes, it induces secretion pressure on beta cell which ultimately leads to degranulation, apoptosis and overt hyperglycaemic state. At this point, the animals rapidly lose their previously accumulated adipose tissue, become ketotic and require insulin to survive. *e.g.*, *db/db* (diabetic) mouse, Zucker diabetic fatty (ZDF) rat, sand rat (*Psammomys obesus*) and obese rhesus monkeys¹¹. The animals with 'brittle' pancreas closely simulate the disease evolution from insulin resistance to progressive beta cell failure/frank hyperglycaemia as in human type 2 diabetes, than the animals with sturdy pancreas. Some of these animals with related phenotype of obesity and insulin resistance such as ZFR, ZDF rats and *ob/ob*, *db/db*, KK and KK-A^y mice would be greatly helpful in identifying factors involved in obesity-induced diabetes (diabesity). Nevertheless, certain non obese diabetic models are also used in the investigation of type 2 diabetes in humans that occur in the absence of obesity which allows the dissociation of confounding obesity factors such as leptin deficiency and/or leptin resistance and other associated hypothalamic factors from diabetes genes and factors [*e.g.*, GK (Goto-Kakizaki) rats, Akita mouse]⁶.

Table I. Classification of type 2 diabetes in animals

| Model category | Type 2 diabetic models | |
|--|--|---|
| | Obese | Non obese |
| I. Spontaneous or genetically derived diabetic animals | <i>ob/ob</i> mouse <i>db/db</i> mouse KK mouse KK/A ^y mouse NZO mouse NONcNZO10 mouse TSOD mouse M16 mouse Zucker fatty rat ZDF rat SHR/N-cp rat JCR/LA-cp rat OLETF rat Obese rhesus monkey | Cohen diabetic rat GK rat Torri rat Non obese C57BL/6 (Akita) mutant mouse ALS/Lt mouse |
| II. Diet/nutrition induced diabetic animals | Sand rat C57/BL 6J mouse Spiny mouse | --- |
| III. Chemically induced diabetic animals | GTG treated obese mice | Low dose ALX or STZ adult rats, mice, <i>etc.</i> Neonatal STZ rat |
| IV. Surgical diabetic animals | VMH lesioned dietary obese diabetic rat | Partial pancreatectomized animals <i>e.g.</i> dog, primate, pig & rats |
| V. Transgenic/knock-out diabetic animals | β_3 receptor knockout mouse Uncoupling protein (UCP1) knock-out mouse | Transgenic or knock out mice involving genes of insulin and insulin receptor and its components of downstream insulin signaling <i>e.g.</i> IRS-1, IRS-2, GLUT-4, PTP-1B and others PPAR- γ tissue specific knockout mouse Glucokinase or GLUT 2 gene knockout mice Human islet amyloid polypeptide overexpressed rat (HIP rat) |

KK, Kuo Kondo; KK/A^y, yellow KK obese; VMH, ventromedial hypothalamus; ZDF, Zucker diabetic fatty; NZO, New Zealand obese; TSOD, Tsumara Suzuki obese diabetes; SHR/N-cp, spontaneously hypertensive rat/NIH-corpulent; JCR, James C Russel; OLETF, Otuska Long Evans Tokushima fatty; GTG, gold thioglucose; ALX, alloxan; STZ, streptozotocin; GLUT-, glucose transporter; IRS, insulin receptor substrate; GK, Goto-Kakizaki; PPAR, Peroxisome proliferator activated receptor, PTP, phosphotyrosine phosphatase; ALS, alloxan sensitive

Table II. Advantages and disadvantages of different categories of type 2 diabetic animal models

| Model Category | Advantages | Disadvantages |
|---|---|---|
| I. Spontaneous diabetic animals | Development of type 2 diabetes is of spontaneous origin involving genetic factors and the animals develop characteristic features resembling human type 2 diabetes | Highly inbred, homogenous and mostly monogenic inheritance and development of diabetes is highly genetically determined unlike heterogeneity seen in humans |
| | Mostly of inbred animal models in which the genetic background is homogeneous and environmental factors can be controlled, allow genetic dissection of this multifactorial disease easy | Limited availability and expensive for the diabetes study Mortality due to ketosis problem is high in case of animals with brittle pancreas (db/db, ZDF rat <i>P. obesus</i> , etc.) and require insulin treatment in later stage for survival |
| | Variability of results perhaps minimum and require small sample size | Require sophisticated maintenance |
| II. Diet/Nutrition induced diabetic animals | Develop diabetes associated with obesity as a result of overnutrition as in diabetes syndrome of human population | Mostly require long period of dietary treatment No frank hyperglycaemia develops upon simple dietary treatment in genetically normal animals and hence become not suitable for screening antidiabetic agents on circulating glucose parameter |
| | Toxicity of chemicals on other body vital organs can be avoided | |
| III. Chemical induced diabetic animals | Selective loss of pancreatic beta cells (alloxan/STZ) leaving other pancreatic alpha and delta cells intact | Hyperglycaemia develops primarily by direct cytotoxic action on the beta cells and insulin deficiency rather than consequence of insulin resistance |
| | Residual insulin secretion makes the animals live long without insulin treatment | Diabetes induced by chemicals is mostly less stable and at times reversible because of the spontaneous regeneration of beta cells. Hence, care must be taken to assess the pancreatic beta cell function during long-term experiments |
| | Ketosis and resulting mortality is relatively less | |
| | Comparatively cheaper, easier to develop and maintain | Chemical produce toxic actions on other body organs as well besides its cytotoxic action on beta cells Variability of results on development of hyperglycaemia is perhaps high |
| IV. Surgical diabetic animals | Avoids cytotoxic effects of chemical diabetogens on other body organs | Involvement of cumbersome technical and post operative procedures |
| | Resembles human type 2 diabetes due | Occurrence of some other digestive |

| Model Category | Advantages | Disadvantages |
|--|---|--|
| | to reduced islet beta cell mass | problems (as a result of part of excision of exocrine portion (deficiency of amylase enzyme) Dissection of alpha islets (glucagon secreting cells) too along with beta cells leading to problems in counter regulatory response to hypoglycaemia Mortality is comparatively higher |
| V. Transgenic/knock out diabetic animals | Effect of single gene or mutation on diabetes can be investigated <i>in vivo</i> Dissection of complex genetics of type 2 diabetes become easier | Highly sophisticated and costly procedure for the production and maintenance Expensive for regular screening experiments |

Various types of animal models of type 2 diabetes derived either spontaneously or induced by treating with chemicals, or dietary or surgical manipulations and combinations there of are summarized in the Table I. Further, category of large number of new animal models developed in the recent years by genetic engineering/molecular biological techniques including transgenic and knock-out mice has initiated a new era in diabetes research. In each category, the animals are further subdivided into models with or without obesity. The models obtained from above categories differ significantly from each other and have their own advantages and disadvantages (Table II), and are useful for the investigation of aetiopathogenesis of diabetes and for testing various NCEs for the treatment of diabetes. Further, the expression and severity of metabolic, hormonal and pathologic abnormalities of diabetes perhaps varies in different animals according to the genetic background, nutrition, age, sex, species and even strain. Generally, the animal models of diabetes exhibit similar characteristic features such as chronic hyperglycaemia, hyper- or normo- or hypoinsulinaemia while the symptoms of clinical diabetes *viz.*, polyuria, polydipsia, polyphagia, lethargy may also appear in several models of diabetes. Apart from the defects in glucose metabolism, the altered lipid metabolism associated with increase in plasma lipid levels is commonly observed in many animal models as in human diabetic patients. In addition, they exhibit various diabetic

complications such as neuropathy, nephropathy, cardiomyopathy, atherosclerosis, hypertension and various others^{7,9,12}. Though some models could be useful for investigation of diabetic complications, the description on the same is beyond the scope of this review.

Spontaneous type 2 diabetic models

Spontaneously diabetic animals of type 2 diabetes may be obtained from the animals with one or several genetic mutations transmitted from generation to generation (*e.g.*, *ob/ob*, *db/db* mice) or by selected from non-diabetic outbred animals by repeated breeding over several generation [*e.g.*, (GK) rat, Tsumara Suzuki Obese Diabetes (TSOD) mouse]. These animals generally inherited diabetes either as single or multigene defects. The metabolic peculiarities result from single gene defect (monogenic) which may be due to dominant gene (*e.g.*, Yellow obese or *KK/A^y* mouse) or recessive gene (diabetic or *db/db* mouse, Zucker fatty rat) or it can be of polygenic origin [*e.g.*, Kuo Kondo (KK) mouse, New Zealand obese (NZO) mouse]¹³. Type 2 diabetes occurring in majority of human being is a result of interaction between environmental and multiple gene defects though certain subtype of diabetes do also exist with well defined cause [*i.e.*, maturity onset diabetes of youth (MODY) due to defect in glucokinase gene] and this single gene defects may cause type 2 diabetes only in few cases.

Therefore, polygenic animals represent the human condition more closely when compared to monogenic animals⁶.

Spontaneous type 2 diabetic obese models

ob/ob mouse: *ob/ob* mouse (obese mouse) (now relabeled as *Lep^{ob}*) is inherited as (monogenic) autosomal recessive mutation on chromosome 6 (obese) in C57BL/6J mouse strain, originating from the Bar Harbor, Jackson laboratory⁷. The mutation in *ob/ob* mice is now identified in leptin gene, which encodes for leptin. Homozygous mutant mice exhibit rapid gain in body weight and may reach the three times of the normal weight of wild type control. There is impaired thermogenesis detectable very early at 10 days. In addition, there is marked hyperphagia and decreased energy expenditure resulting in increased carcass lipid with obvious obesity by approximately 4 wk. In addition to obesity (pear shaped body), it is characterized by diabetes like syndrome of hyperglycaemia, mild impaired glucose tolerance, severe hyperinsulinaemia, sub fertility and impaired wound healing. Hyperglycaemia is only transient (subsiding around 14 to 16 wk) on the C57BL/6J background. However, when *ob* gene is expressed on C57BL/KS background, mice become severely diabetic with regression of islets and early death^{4,7}. The obesity is characterized by both hypertrophy and

hyperplasia of pancreatic islets¹². Hyperinsulinaemia does not develop until after the increase in body weight and probably the result of it. Insulin resistance is associated with hepatic glucose overproduction, increased activity of gluconeogenic enzymes, decreased activity of glycolytic and glycogen synthesis enzymes and increased lipogenesis in the liver. The circulating glucose concentration is almost maintained normally in adult C57BL/6J (*ob/ob*) mouse due to sustained hyperinsulinaemia. At molecular level, insulin resistance in *ob/ob* mice appears to be due to diminished insulin binding to receptors, impaired insulin receptor (IR) autophosphorylation, and reduced signal transduction⁹.

Leptin, a 16 kDa protein expressed predominantly in adipose tissue of normal mice, is missing from these mice homozygous for the mutation 'obese'. Leptin functions as a hormone keeping the brain apprised about the amount of body fat and activating centers involved in regulating feed intake and energy expenditure. Hence the major deficiency of satiety factor leptin in these mice significantly alters feeding behaviour, metabolism, and endocrine function, resulting in hyperphagia, decreased energy expenditure and obesity. Levels of neuropeptide Y (NPY), a potent appetite stimulant (orexigenic) peptide are high in these mice, possibly a result of

Table III. The doses of various chemical diabetogens in different species

| Chemicals | Species | Dose (s) (in mg/kg) | References |
|----------------|----------|---------------------|-----------------------|
| Alloxan | Rat | 40-200 (iv or ip) | 9, 25, 74, 75 |
| | Mice | 50-200 (iv or ip) | 9, 74, 76 |
| | Rabbit | 100-150 (iv) | 9, 25, 77 |
| | Dog | 50-75 (iv) | 25, 74 |
| Streptozotocin | Rat | 35-65 (iv or ip) | 9, 80, 74, 81, 84, 85 |
| | Mice | 100-200 (iv or ip) | 9, 80, 74, 77, 81 |
| | Hamster | 50 (ip) | 86 |
| | Dog | 20-30 (iv) | 74, 77 |
| | Pig | 100-150 (iv) | 77, 87, 88 |
| | Primates | 50-150 (iv) | 77, 88, 89 |

iv, intravenous; ip, intraperitoneal

leptin deficiency. The lack of leptin leads to increased circulating cortisol levels (hypercorticism) which may contribute to a state of muscle insulin resistance and adrenalectomy of *ob/ob* mouse lowers blood glucose and partially restores insulin sensitivity⁹. Though severe obesity due to leptin deficiency has been documented in humans, the incidence is extremely low. Cloning of *lep* gene has made possible the production of recombinant leptin. Injection of leptin into obese mice was shown to reduce body weight gain, decrease food intake, increase energy expenditure and improves insulin sensitivity¹⁴. Since this model is severely obese, hyperinsulinaemic and insulin resistant throughout its life, the agents that decrease the body weight and improves peripheral insulin sensitivity like insulin sensitizers, antiobesity and some other antihyperglycaemic agents have been largely tested and shown to produce antidiabetic activity^{2,15-19}.

db/db mouse: The *db/db* (diabetic) mouse (now relabeled as *lepr^{db}*) is originally derived from an autosomal recessive mutation on chromosome 4 in mice of C57BL/KsJ strain originating from Bar Harbor, Maine⁷. The mutation in this diabetic animal was traced to *db* gene, which encodes for the leptin receptors. These mice are spontaneously hyperphagic insulin oversecretors becoming obese, hyperglycaemic, hyperinsulinaemic and insulin resistant within first month of age and develop hypoinsulinaemia, hyperglycaemia later with a peak between 3-4 months of age. Animals then exhibit ketosis, progressive body weight loss and do not survive longer than 8-10 months. In *db/db* mice, lack of leptin receptors result in similar hypothalamic disturbances and NPY abnormalities as in *ob/ob* mice, but the leptin is still produced and NPY induced hyperinsulinaemia plus hypercorticism result in *ob* gene hyperexpression and hyperleptinaemia. Unlike *ob/ob* mice, exogenous administration of leptin fails to elicit effect on food intake and body weight as there is defect in leptin receptor⁹. *db/db* mice have been commonly and extensively used for the investigation of type 2 diabetes/diabetic

dyslipidaemia and screening variety of agents such as insulin mimetic and insulin sensitizers^{2,15,18-23}.

KK mouse: KK (Kuo Kondo) mouse is polygenic model of obesity and type 2 diabetes produced by selective inbreeding for the large body size in Japan, also named as Japanese KK mouse^{6,12}. These animals are hyperphagic, hyperinsulinaemic, insulin resistant and show moderate obesity by 2 months of age, which attains maximum at 4-5 months. Insulin resistance precedes the onset of obesity. The increase in pancreatic insulin content is associated with increase in number and size of pancreatic islets but histologically degranulation of beta cells and hypertrophy of islets are found^{24,25}. There is selective failure of insulin to suppress gluconeogenic pathway, while exerting its inductive effect on glycolysis and lipogenesis as seen in hepatic insulin resistance of *db/db* mouse.

KK/A^y mouse: KK/A^y mouse (also known as Yellow KK obese mouse) carries both lethal yellow obese (A^y) and diabetic gene unlike KK mouse where it carries only diabetic gene. Mice homozygous for the yellow spontaneous mutation (A^y) die before implantation or shortly thereafter. KK/A^y mouse is heterozygous which shows severe obesity, hyperglycaemia, hyperinsulinaemia and glucose intolerance by 8 wk of age. The strain KK/A^y mice maintained at Upjohn colony (KK/Upj-A^y/J) are now available from Jackson Laboratory, Bar Harbor, USA, and serve as a good model for obesity and type 2 diabetes and for screening various classes of antidiabetic agents^{2,18,19,26,27}. Hyperphagia and obesity in young is more pronounced in males than in females. It is not clear whether hyperphagia found in these mice is associated with reduced hypothalamic (glucagon like peptide) GLP-1 content⁶. Studies using isolated adipocytes indicate that tissue responsiveness to insulin is decreased progressively from 5 wk of age. Histo- and immunochemical studies show that pancreatic islets are hypertrophied and beta cells are degranulated²⁵. These findings suggest that principal cause of diabetes in these mice is insulin resistance which may

be due to defects in both insulin receptor and post receptor signaling systems, including glucose uptake, pentose pathways, and impaired insulin sensitive phosphodiesterase in fat cells⁹.

New Zealand Obese (NZO) mouse: The NZO strain is a polygenic model of obesity and diabetes obtained by selective inbreeding over several generation with the parents selected for their agouti coat color. It exhibits a polygenic syndrome of hyperphagia, obesity, mild hyperglycaemia, hyperinsulinaemia, impaired glucose tolerance and insulin resistance. Body weight rises rapidly during first 2 months of life, due to hyperphagia. Hyperleptinaemia and leptin resistance which may account for hyperphagia, have been reported in NZO mice⁹. Hyperglycaemia and impaired glucose tolerance increase continuously with advancing age of animals. There is a marked hyperplasia and hypertrophy with islets composed of up to 90 per cent of beta cells⁴. Reduced glycogen synthase activity in liver has been considered as a primary early defect in causation of diabetes²⁸. The mice is shown to have hepatic insulin resistance from an early age and have increased gluconeogenesis and hepatic glucose production associated with the abnormality in the regulation of gluconeogenic enzyme fructose-1,6-bisphosphatase^{29,30}. These mice have high prevalence of autoimmune disorder and become a useful model for studying relationship between autoimmunity, obesity and diabetes²⁵.

NONcNZO10 mouse: It is a recombinant congenic new mouse strain model of type 2 diabetes developed by introgressing 5 genomic intervals containing NZO/H1Lt (NZO) diabetogenic quantitative trait loci onto the non obese non diabetic (NON/Lt or NON) genetic background at Jackson laboratory, Maine³¹. Whereas parental NZO males exhibit the unwanted phenotypes of hyperphagia, morbid obesity, poor fertility, and a variable frequency of hyperglycaemia, NONcNZO10 males are not hyperphagic, develop more moderate level of obesity, and reproduce normally. They weigh more than NON males, but significantly less than the NZO males. Despite the reduced rate of weight gain compared to NZO, all

NONcNZO10 males develop chronic hyperglycaemia by 12-20 wk. Pancreatic islets show the similar atrophic changes as seen in diabetic NZO males. Increased serum triglycerides and liver steatosis are observed. Females are diabetes resistant and can serve as normoglycaemic control. Unlike *ob/ob* and *db/db* mice which are monogenic and exhibit a morbid obesity, these mice are polygenic, not hyperphagic, have a normal leptin/leptin receptor axis, do not exhibit hypercorticism, and show no obvious thermoregulatory defects as seen in humans with obesity/diabetes syndrome³¹. Unlike parental NZO mouse, this model has gained lot of attention in the recent days for the investigation of diabetes as well as in drug testing for the treatment of type 2 diabetes³².

TSOD mouse: By selective breeding of obese male mice of ddY strain, Tsumara and Suzuki described the two inbred strains, one with obesity with increase in urinary glucose named TSOD (Tsumara Suzuki Obese Diabetes) and other without them (TSNO, Tsumara Suzuki Non Obese). TSOD mouse is of polygenic origin and characterized by polydipsia and polyuria at about 2 months old only in male mice followed by hyperglycaemia and hyperinsulinaemia. Following these symptoms, obesity gradually develops until about 12 months. Pancreatic islets of TSOD male mice are found hypertrophic without any signs of insulinitis or fibrous formation^{33,34}. It has been shown that the reduced insulin sensitivity in diabetic TSOD mice is due, at least in part, to the impaired glucose transporter (GLUT4) translocation by insulin in both skeletal muscle and adipocytes³⁵.

M16 mouse: M16 mouse is a new model for obesity and type 2 diabetes which results from long-term selection for 3 to 6 wk weight gain from an Institute of Cancer Research, London, UK (ICR) base population. M16 mice exhibit early onset of obesity and are larger at all ages characterized by increased body fat percentage, fat cell size, fat cell numbers, and organ weights. These mice also exhibit hyperphagia, accompanied by moderate obesity, and are hyperinsulinaemic, hyperleptinaemic and

hypercholesterolaemic relative to ICR. Both M16 males and females are hyperglycaemic relative to ICR, with 56 and 22 per cent higher fasted blood glucose levels at 8 wk of age. M16 mice represent an outbred animal model to facilitate gene discovery and pathway regulation controlling early onset polygenic obesity and type 2 diabetic phenotypes. Phenotypes prevalent in the M16 model, with obesity and diabetes exhibited at a young age, closely mirror current trends in human populations³⁶.

Zucker fatty rat: The spontaneous mutation 'obese' (fatty) was found in the rat stock of Sherman and Merck, by Zucker, Harriet Bird Memorial Laboratory, Stow, Massachusetts, USA in 1961. The Zucker (*fa/fa*) fatty or obese rat (now labeled as *Lep^{fa}*) results from the simple autosomal recessive (*fa*) gene on chromosome 5. It is characterized by hyperphagia and early onset of obesity which appear at 4 wk of age along with increased growth of subcutaneous fat depot. It is also associated with mild hyperglycaemia, insulin resistance, mild glucose intolerance, hyperlipidaemia, hyperinsulinaemia and moderate hypertension^{7,37}. The hyperphagia seen in this rat has been attributed to hypothalamic defect in leptin receptor signalling⁹. The other hormonal changes in Zucker rats include hypersomatostatinaemia despite hyperglycaemia, particularly in older animals. There are decreased growth hormone and prolactin levels⁷.

It is reported that abnormal glucose tolerance found in these rats is due to the metabolic defects in hepatic organ, as glucose clearance in obese rat is normal⁶. It is useful as a model for human obesity, type 2 diabetes associated with type IV hyperlipidaemia (increased VLDL and triglyceride levels in the circulating blood) and hypertension. This model has been mostly used for screening the effects of different insulin sensitizing and antiobesity agents and in few cases for the testing the potentiators of insulin secretion or incretin mimetic agents^{2,18,19,38,39}.

Zucker diabetic fatty rat: It is a substrain of ZFR selectively inbred for hyperglycaemia and is highly

useful for the investigation of mechanism of type 2 diabetes. Unlike ZFR, male Zucker diabetic fatty (ZDF) rat progresses to frank diabetes due to failure to compensate adequately for insulin resistance. It is less obese but more insulin resistant than ZFR⁵. Males are more prone to the development of diabetes usually at 7-10 wk after birth. This model is presently available with Charles River laboratories, Indianapolis, IN, USA. However, female littermates are obese, insulin resistant but do not develop diabetes and serve as control. In contrast to *fa/fa* rats, the ability to over secrete insulin to compensate peripheral insulin resistance is limited, and the beta cells are brittle, easily succumb to over secretion pressure. Studies on cell turnover in these animals suggest that the primary defect lies not in an ability of beta cells to proliferate but rather in an enhanced rate of apoptosis⁴⁰. It exhibits impaired insulin secretory beta cell response to glucose while it remains intact to non glucose secretagogues like arginine (ARG), a phenomenon similar to human type 2 diabetic patients. Downregulation of beta cell GLUT-2 transporters coupled with impaired insulin synthesis has been reported to be responsible for hyperglycaemia in ZDF rats. Decreased glucose transport activity associated with decreased GLUT-4 levels is observed in adipose tissue and skeletal muscles of ZDF rats. The previous study demonstrated the deleterious phenomenon of lipotoxicity of high plasma free fatty acid (FFA) and beta cell triglyceride levels on beta cell function in ZDF rats⁴¹. This ZDF rat has been most commonly used for investigating the mechanisms associated with insulin resistance and beta cell dysfunction in type 2 diabetes, as well as for testing insulin sensitizers insulinotropic and various other agents^{2,19,38,39}.

SHR/N- cp rat: SHR/N-*cp* rat (spontaneously hypertensive rat/NIH-corpulent) is derived by inbreeding of SHR/N strains, at the National Institute of Health (NIH), Bethesda, Maryland, USA, a genetic model of obesity, type 2 diabetes with hypertension. SHR/N-*cp* rats if homozygous for corpulent gene (*cp*), males exhibit hyperphagia and early onset of

obesity, normal or slight hyperglycaemia, dyslipidaemia, profound hyperinsulinaemia, hyperleptinaemia, insulin resistance, impaired glucose tolerance and essential hypertension⁴². This SHR/N-*cp* rats is highly useful for investigating obesity associated type 2 diabetes and also for studying the influence of dietary carbohydrate on the development of diabetes in certain genetically predisposed carbohydrate sensitive individuals.

JCR/LA -cp rat: Backcrosses of LA/N-*cp* males with hooded rat species produced an outbred substrain JCR (James C Russel):LA-*cp* with only 3 per cent contribution of the *SHR* gene but the presence of *fa* allele. The homozygous dominant (+/+) and heterozygous (+/*cp*) rats are metabolically unaffected. The animals which are recessive for the gene (*cp/cp*) exhibit extreme metabolic profile including insulin resistance, hyperinsulinaemia, pancreatic beta cell hyperplasia, obesity, glucose intolerance and severe hyperlipidaemia. The *cp* gene encodes a stop codon in the leptin receptor producing non functional receptor protein. Leptin receptor deficient states along with hypothalamic dysregulation of peptides contribute to hyperphagia and other metabolic abnormalities in these corpulent rats. The major drawback of this rat as pure model of diabetes is that it is normoglycaemic when fasted. However, the principal attraction of JCR/LA-*cp* rat as research model is its development of atherosclerotic and myocardial lesions in association with the Syndrome-X metabolic profile⁴³. The distinct feature of this animal is the vasculopathy progresses inherently without any dietary cholesterol and high fat diet interventions. This model is extensively studied for the pharmacological and dietary intervention prior to the onset of cardiovascular lesions or hyperinsulinaemia to determine the efficacy for preventing or slowing the occurrence of cardiovascular lesions.

OLETF rat: OLETF (Otsuka Long Evans Tokushima Fatty) rat with mild obesity was obtained from the selective breeding of the spontaneous diabetic rats from the outbred colony of Long Evans rats

maintained in Otsuka pharmaceuticals, Tokushima, Japan. This polygenic rat develops diabetes later in life at around 18-25 wk old and inherited mostly in males. OLETF rats exhibit innate polyphagia, mild obesity, hyperinsulinaemia, hypertriglyceridaemia, hypercholesterolaemia, chronic course hyperglycaemia and impaired glucose tolerance at about 16 wk of life⁴⁴. Evidences suggest that defects in the beta cell proliferation *per se* is responsible for the development of diabetes in OLETF rats since 70 per cent pancreatectomized animals exhibit sustained hyperglycaemia due to poor capacity of pancreatic islet regeneration after surgery and the treatment with nicotinamide (NAD) corrects hyperglycaemia by increasing beta cell proliferation⁴⁵. In the recent years, OLETF rat model has been extremely used in pharmacological research while testing for a number of antidiabetic and antihypertensive drugs⁴⁶⁻⁴⁸.

Obese rhesus monkey (Macaca mullata): Obese rhesus monkey, an excellent non rodent model develops obesity, hyperinsulinaemia and insulin resistance when maintained on *ad libitum* laboratory diet, which gradually progresses to necrosis of beta cells, severe fall in insulin levels and overt hyperglycaemia over a period of several years⁴⁹. Unlike conventional rodent models, the final secretion loss is interestingly associated with deposition of amylin/amyloid in beta cells and the development of diabetic complications similar to human type 2 diabetes^{4,9,49}. Pioglitazone has been demonstrated to improve insulin resistance in obese rhesus monkeys⁵⁰.

Spontaneous type 2 diabetic non obese models

Cohen diabetic rat: The Cohen diabetic rat is an exceptional genetically derived experimental model of diet induced type 2 diabetes that reproduces many features of the disease in humans. Its most outstanding and distinctive feature is that it expresses genetic susceptibility (sensitivity and resistance) to a carbohydrate-rich diet, a central feature of type 2 diabetes⁵¹. Recently, the Cohen rat strain was newly inbred and metabolic phenotypes of this rebred colony of CDs (Cohen diabetic sensitive) and CDr (Cohen

diabetic resistant) rats and their genetic makeup render the Cohen diabetic rat a useful experimental model that is highly suitable for studying the interaction between nutritional-metabolic environmental factors and genetic susceptibility for the development of type 2 diabetes and also distinctively useful for investigating the effect of sex on the expression of the diabetic phenotype⁵¹.

GK rat: The GK (Goto-Kakizaki) rat, a polygenic model of type 2 diabetes was established by Goto and his collaborators through selective inbreeding of Wistar rats with abnormal glucose tolerance repeated over several generations in Japan in 1973. It is characterized by non obesity, moderate but stable fasting hyperglycaemia, hypoinsulinaemia, normolipidaemia, impaired glucose tolerance which appears at 2 wk of age in all animals and an early onset of diabetic complications^{52,53}. In the adult GK rats, total pancreatic beta cell mass is decreased by 60% along with same degree of decrease in pancreatic insulin stores. The defective beta cell mass and function in the GK rat model may also result from inadequacy of pancreatic growth factors necessary for the growth and development of fetal pancreatic cells during gestation and secondary (acquired) loss of beta-cell differentiation due to chronic exposure to hyperglycaemia (glucotoxicity) indicating that type 2 diabetes is not simply dependant on genetic factors, but probably involve transgenerational epigenetic responses^{54,55}. In addition to the defects in beta cells, impaired insulin sensitivity in the liver, skeletal muscle and adipose tissues has also been reported. Impaired insulin secretion and hepatic glucose overproduction (hepatic insulin resistance) are early events in diabetic GK rats mostly contributing to development of hyperglycaemia rather than the peripheral (muscle and adipose tissue) insulin resistance⁵⁶. GK rat is one of the best characterized animal models used for studying the relation of changes in beta cell mass and occurrence of type 2 diabetes and diabetic complications (particularly diabetic nephropathy). However, only very few studies on drug testing using this model have been reported in the literature⁵⁷.

Torri rat: This is a new spontaneously diabetic non obese rat from the Sprague-Dawley rat strain established recently in 1997 at Torri Pharmaceutical Co, Japan⁵⁸. It is characterized by glucose intolerance, hyperglycaemia, hypoinsulinaemia and hypertriglyceridaemia. Histologically, hemosiderin deposition and marked fibrosis of pancreatic islets are observed in diabetic rats. Torri rats are able to survive for longer duration without insulin treatment and hence more useful for studies on diabetic complications. The distinct characteristics are ocular complications, cataract and retinopathy with fractional, retinal detachment, fibrous proliferation and massive haemorrhage at 70-77 wk of age⁵⁸. The genetic analysis for diabetes in Torri rat identified (Quantitative trait loci) three distinctive QTLs for glucose intolerance which is highly associated with development of diabetes⁵⁹.

Non obese mutant C57 BL/6 (Akita) mouse: The non obese mutant mouse (Akita mouse) has been derived from the colony of C57 BL/6 (B6) in Akita (Japan) and now commercially available for research at Jackson Laboratory, Bar Harbor. The *Ins2* gene is the mouse homologue of human preproinsulin gene. Mice possess another active insulin gene, *Ins1*, which lacks an intron present in the C-polypeptide-encoding region. The Akita (*Ins2^{Akita}*) spontaneous mutation (commonly referred as Mody) is an autosomal dominant mutation in the insulin II gene (*Ins2*)⁶⁰. *Ins2^{Akita}* mutation disrupts normal insulin processing and causes a failure in secretion of mature insulin, which results in early development of hyperglycaemia. It is characterized by hyperglycaemia, hypoinsulinaemia, polydipsia and polyuria, beginning around 3-4 wk of age. Obesity or insulinitis does not accompany diabetes. Histologically, at 4-35 wk of age, there is a reduction in active pancreatic beta cell density without insulinitis and islets release very little mature insulin. These mutant mice respond well to exogenously administered insulin. The mice with reduced beta cell mass and absence of beta cell autoimmunity serve as an excellent substitute for mice made diabetic by treatment with certain chemical diabetogens for islet transplantation studies⁶¹.

ALS/Lt mouse: Alloxan susceptible (ALS) new mouse model is produced by inbreeding outbred CD-1 mice (a commercial stock of ICR mice from which inbred NSY and NON mouse are developed), with selection for susceptibility to alloxan (ALX), a generator of highly reactive oxygen free radicals and a potent beta-cell toxin. Initially, the type 2 diabetes predisposition of ALS mouse was recognized by congenic analysis of the yellow mutation (*Ay*) at the agouti locus on chromosome 2. Indeed, in ALS/Lt (a substrain maintained at Jackson Laboratory, Bar Harbor) mice, hyperinsulinaemia and impaired glucose tolerance develop spontaneously between 6 and 8 wk of age in alloxan-untreated males. This mouse model with reduced ability to diffuse free radical stress is of obvious interest because free radical-mediated damage is implicated in the pathogenesis and complications of both type 1 and type 2 diabetes⁶².

Dietary or nutrition induced type 2 diabetic models

Some of the animal models exist in which diabetes is induced neither by chemicals nor by genetic defect. Sand rat, Tuco-Tuco and Spiny mouse are important models of nutritionally induced obesity and type 2 diabetes⁷.

Sand rat: Psammomys obesus (P. obesus; Sand rat) remains normal in its natural habitat but develop obesity and diabetes in captivity when fed on standard laboratory chow (high energy diet) instead of its usual low energy vegetable diet (mainly of *Atriplex*)^{11,41}. Initially, the sand rats develop hyperphagia, obesity, hyperinsulinaemia, glucose intolerance with pancreatic islet cells remain intact followed by beta cell degeneration and necrosis resulting in profound insulin deficiency and overt diabetes and ketosis ultimately leading to death of animal⁶³. In this animal model insulin, even at remarkably high levels, is not sufficient to overcome muscle insulin resistance as noted by decreased 2 deoxyglucose uptake and diminished GLUT-4 protein and restrain hepatic gluconeogenesis as evidenced by elevated phosphoenolpyruvate carboxykinase (PEPCK) activity. Part

of the insulin inefficiency is also due to increased proinsulin: insulin ratio in pancreatic beta cells as observed in human type 2 diabetic patients and resulting in elevated proinsulin and relative hypoinsulinaemia in circulation⁶⁴. In later stages of development, these animals show decrease in adipose tissue, depletion of beta cell granules, apoptosis and develop ketoacidosis. Then, they require insulin support for survival⁴¹.

Sand rats are used extensively in the drug testing such as protein tyrosine phosphatase inhibitor⁶⁵ and glucagon like peptide-1 (GLP-1) analogues⁶⁶. A novel gene that encodes for a small protein named 'beacon' has been discovered in the hypothalamus of *P. obesus* by Collier and co-workers⁶⁷. Overexpression of beacon mRNA gene is positively correlated with percentage of body fat⁶⁷. Further, a novel protein named 'Tanis' expressed in the liver of *P. obesus* has been shown to have mechanistic link among type 2 diabetes, inflammation, and cardiovascular disease⁶⁸. Very recently, a new candidate gene, the mitochondrial rhomboid protease, presenilins-associated rhomboid-like protein (PSARL) in skeletal muscle is found to be associated with insulin resistance and type 2 diabetes. Expression of PSARL has been reduced in skeletal muscle of diabetic *P. obesus*, and restored after exercise training to successfully treat the diabetes⁶⁹. Thus, sand rats are studied extensively and serve as an excellent polygenic model for the study of diabetes syndrome^{41,64}.

C57BL/6J mouse: Type 2 diabetic model by simply feeding high fat feed to non obese, non diabetic C57BL/6J mouse strain was initially developed in Japan and is now available at Jackson laboratory, Bar Harbor. It is characterized by marked obesity, hyperinsulinaemia, insulin resistance and glucose intolerance⁷⁰. In addition, they exhibit marked fasting as well as basal hyperglycaemia in contrast to normal basal glucose level seen in C57BL/6J (ob/ob) mice. These mice are demonstrated to develop peripheral leptin resistance. They manifest most of the characteristic features of the patients with genetic

predisposition to develop type 2 diabetes when they become obese. This animal model represents both genetic and environmental risk factors in contrast to C57BL/6J (*ob/ob*) mouse in which onset of symptoms is highly genetically determined. Further, its usefulness for drug testing has been reported in the literature as these mice treated with orally active inhibitor of dipeptidyl peptidase-IV (LAF237) are shown to have normalized glucose tolerance in association with augmented insulin secretion⁷¹.

In addition, *Acomys calirinus* (spiny mouse), a small rodent living in semi desert areas of eastern Mediterranean, in fact are low insulin secretors. However, when they are placed in captivity on high energy rodent lab chow, they gain weight and exhibit marked pancreatic beta cell hyperplasia, hypertrophy and increased pancreatic insulin content in comparison to other animal models yet, the plasma insulin response to glucose as well as to other secretagogues is impaired suggesting a impairment in hormone release mechanisms¹². They are reported to accumulate insulin in beta cells, which may disintegrate and produce insulin-deficiency. These animals develop frank hyperglycemia with glucosuria leading to fatal ketosis^{11,25}. *Ctenomys talarum* (Tuco-tuco) is another species found in natural environment which exhibit similar characteristic features of sand rat as well as spiny mice when fed on high energy rodent diet²⁵.

Chemically induced type 2 diabetic models

Chemically induced models of diabetes are common in elucidating the possible role of environmental factors involved in the endocrine pancreatic destructive processes and subsequent development of diabetes.

Goldthioglucose obese diabetic mouse: Type 2 diabetes with obesity is induced in mice by goldthioglucose (GTG) (150-350 or 200 mg/kg, ip) injection. Mice gradually develop obesity, hyperinsulinaemia, hyperglycaemia, insulin resistance over a period of 16- 20 wk after GTG

injection⁷². The GTG is transported in particular to the cells of ventromedial hypothalamus (VMH) and causes necrotic lesions, which subsequently is responsible for the development of hyperphagia and obesity. It also shows increased body lipid and hepatic lipogenesis and triglyceride secretion, increased adipose tissue lipogenesis and decreased glucose metabolism in muscle, abnormalities that are qualitatively similar to genetically obese mice (*ob/ob*). In addition, it exhibits many molecular defects in relation to insulin signaling pathways⁷³. However, it is disadvantageous as it takes very long time to develop obesity/diabetes and the number of mortality following GTG injection is extremely high which limits its use in diabetes research.

Chemical type 2 diabetic non obese models

Alloxan and streptozotocin- induced adult diabetic animals: Since the initial findings in 1943 of alloxan (ALX) induced beta cell necrosis in rabbits, this compound has long been used for inducing experimental diabetes. Alloxan is a uric acid derivative and is highly unstable in water at neutral pH, but reasonably stable at pH 3. ALX acts by selectively destroying the pancreatic beta islets leading to insulin deficiency, hyperglycaemia and ketosis⁷⁴. ALX causes diabetes in many rodent and non rodent animals and is most preferably used in case of rabbit because of the relative ineffectiveness of streptozotocin (STZ) in rabbits for induction of diabetes and development of well characterized diabetic complications⁷⁴⁻⁷⁷. However, guineapig and recently musk shrew have been reported to be resistant to the action of ALX due to certain unclear mechanisms^{4,74,78}. Because of its low stability, relatively very shorter half-life (less than 1 min) and acidic nature of solution, intravenous route of administration of ALX is preferred. The hypoglycaemic phase may be quite severe and therefore ALX should not be given to fasted animals. The ALX treated animals exhibit severe hyperglycaemia, glucosuria, hyperlipidaemia, polyphagia, polydipsia and other symptoms of uncontrolled diabetes and do also develop various

complications such as neuropathy, cardiomyopathy, well marked retinopathy and others. ALX is disadvantageous as the percentage incidence of diabetes is quite variable and is not proportionately related to increasing doses of ALX⁷⁷. Further, the incidence of ketosis and resulting mortality is high. The reversal of hyperglycaemia due to pancreatic regeneration is early and common in case of ALX treated animals. Because of these limitations, ALX is now almost replaced by STZ for induction of diabetes in laboratory animals.

Streptozotocin is an antibiotic derived from *Streptomyces achromogenes* and structurally is a glucosamine derivative of nitrosourea. Rakieta and his associates⁷⁹ first demonstrated the diabetogenic property of STZ in dogs and rats in 1963. Like ALX, it causes hyperglycaemia mainly by its direct cytotoxic action on the pancreatic beta cells^{80,81}. The evidences are accumulating on the mechanisms associated with diabetogenicity of STZ. Its nitrosourea moiety is responsible for beta cell toxicity, while deoxyglucose moiety facilitates transport across the cell membrane. Like ALX, the involvement of free radicals generation and resulting alteration of endogenous scavengers of these reactive species have been reported in STZ diabetogenicity. Further, STZ causing alkylation or breakage of DNA strands and a consequent increase in the activity of poly-ADP-ribose synthetase, an enzyme depleting NAD in beta cells finally leading to energy deprivation and death of beta cells is reported. These hypotheses have been confirmed by different studies in which the administrations of various chemicals such as antioxidants (superoxide dismutase; SOD), free radical scavenger (alpha-phenyl-tert-butyl nitron), NAD and poly ADP-ribosyl synthase inhibitors, concomitantly or before STZ injection have been shown to either prevent or lessen the severity of the induction of diabetes, respectively^{80,82}.

There are wide variety of reports available in the literatures on doses and development of hyperglycaemia with STZ since the susceptibility of animals to STZ appear to depend on age, species

and even within strain⁸³ (Table III). STZ is a preferred agent to induce experimental diabetes since it has some advantages over ALX such as, relatively longer half-life (15 min), sustained hyperglycaemia for longer duration and the development of well characterized diabetic complications with fewer incidences of ketosis as well as mortality⁸⁰. ALX and STZ diabetic animals are most widely used for screening the compounds including natural products for their insulinomimetic, insulinotropic and other hypoglycaemic/antihyperglycaemic activities^{18,20,38,84-85,90-93}.

Recently, a new animal model of type 2 diabetes has been produced by combination of STZ and NAD administration in adult rats⁹⁴. The rats administered NAD (230 mg/kg, ip) 15 min before STZ (65 mg/kg, iv) has been shown to develop moderate and stable non-fasting hyperglycaemia without any significant change in plasma insulin level. As NAD is an antioxidant which exerts protective effect on the cytotoxic action of STZ by scavenging free radicals and causes only minor damage to pancreatic beta cell mass producing type 2 diabetes. Therefore, this model is found to be an advantageous tool for investigation of insulinotropic agents in the treatment of type 2 diabetes⁹⁴. Recently, moderate insulin deficiency and type 2 diabetes was developed in a nonrodent model of Gottingen pig by combination of STZ and NAD, which provides good opportunity to investigate diabetes in much closely similar pathophysiological situation as in human⁹⁵.

Neonatal streptozotocin diabetic rats: Unlike the injection of single high dose of STZ, which can produce type 1 diabetes in adult rats, STZ when injected neonatally or immediately after birth, rats develop type 2 diabetes in the adult age. Single injection of STZ at the dose range of 80-100 mg /kg of STZ (iv or ip or sc) to one or two or five day old Wistar or Sprague-Dawley neonatal rats has been reported to produce type 2 diabetic conditions^{96,97}. The neonatal STZ rats are considered to be better tools for the elucidation of the mechanisms associated with regeneration of the beta cells, the

functional exhaustion of the beta cells and the emergence of defects in insulin action^{96,97,98}. Some investigators have also developed neonatal type 2 diabetic models by injecting ALX (200 mg/kg, ip) to male neonatal rats at age of 2, 4 or 6 day after birth⁹⁹ and found to be much useful for the investigation of long term complication of type 2 diabetes.

Thus diabetes produced by the injection of ALX/STZ to animals (adult or neonatal) result mainly from reduction in beta cell mass and consequent insulin deficiency. However, some investigators have attempted to replicate the disease process that naturally occurs in human beings from the progression of insulin resistance to type 2 diabetes in outbred animals. It has been achieved either by injecting chemicals (STZ) into animals which are genetically insulin resistant in its background (*e.g.*, SHR, ZFR)¹⁰⁰ or by combination of diet [high fat (HFD) or high fructose diet] plus STZ treatment. Feeding of above special diets produces hyperinsulinaemia and insulin resistance initially followed by treatment with STZ that causes the beta cell damage and frank hyperglycaemia in the presence of almost absolute normal insulin circulating concentrations in nongenetic, outbred animals such as rats^{101,102} and mice^{103,104}. Recently, we developed a novel type 2 diabetic rat model in our laboratory by combination of short term HFD feeding followed by low dose of STZ (35 mg/kg, ip) treatment¹⁰⁵. It is unique and different from other combination rat models since the dose of STZ selected causes diabetes only in HFD-fed insulin resistant rats where as it fails to induce the same in normal control rats resembling the situation in humans with risk factors of obesity and insulin resistance to be more prone to develop type 2 diabetes than others without them. These rats are not insulinopenic and further responsive to the actions of both insulin sensitizing (pioglitazone) as well as insulinotropic (glipizide) agents. The beta cell responsiveness to insulinotropic agents and the glucose lowering effects of insulin sensitizers is distinctly different and advantageous from neonatal-

STZ and high dose STZ treated adult diabetic rats and could be exploited for screening the above type of agents. In addition, these HFD-fed, low dose STZ-treated rat model interestingly exhibits stable, long lasting hyperglycaemia and the symptoms of type 2 diabetes like polyuria, polydipsia, and polyphagia and diabetic complications such as hypertension. These nongenetic type 2 diabetic rats may be good alternative and cost-effective as compared to genetic models for the investigation as well as regular screening experiments.

Surgical type 2 diabetic models

This method consists of complete or partial pancreatectomy in animals used for the induction of type 1 or type 2 diabetes, respectively. Historically, the diabetic dog model discovered by Oskar Minkowski through surgical complete pancreatectomy has been considered to be the first animal model of diabetes and is rarely now used for the investigation⁸⁰. However, partial pancreatectomy and/or combination methods on animals particularly non rodents are at times utilized in the diabetes investigation for some specific studies as described below.

Non obese partial pancreatectomized diabetic animals: Partial pancreatectomy in animals performed as 70 or 90 per cent (usually 90%) dissection of pancreas has been reported in various animal species mostly in dogs, pigs, rabbit and also rats^{9,106,107}. It does not cause severe form of diabetes and is characterized by moderate hyperglycaemia with neither reduction in body weight nor reduction in plasma insulin levels. The 90 per cent partially pancreatectomized rats also show defect or selective impairment to glucose stimulated insulin release but remain intact to other insulin secretagogues *viz.*, ARG, isoproterenol like neonatal STZ rats. This finding from these partial pancreatectomized animals supports the notion that simply reduction in pancreatic beta cell mass itself may not be responsible for the glucose intolerance as seen in neonatal STZ rats¹⁰⁸. These partial

pancreatectomized animals are reported to develop hyperglycaemia and insulin resistance. Improvement of hyperglycaemia and insulin resistance is observed by administration of insulin or phlorizin, an inhibitor of renal glucose reabsorption⁹⁷. However, better degree of glycaemia or stable form of diabetes for long duration can be achieved by the combination of partial pancreatectomy with chemicals *viz.*, ALX and STZ injection in animals such as dog, pig, monkey and others^{77,109}. Recently, yet another model on stable form of type 2 diabetes has been produced by combination of 50 per cent partial pancreatectomy along with NAD (350-mg/kg) and STZ (200 mg/kg) treatment in BALB/c mice¹¹⁰. There are some advantages in combination procedure as it minimizes the risk of unnecessary adverse effect of chemicals on body following its single high dose as well as reduces post-operative interventions following pancreatectomy. Pancreatic regeneration following pancreatectomy has been demonstrated in many animal models including humans¹¹¹. These partially pancreatectomized models alone or in combination are highly useful in the investigation of transplantation of pancreatic grafts or islets. Further this helps in identification of factors involved in islet regeneration (such as “reg” gene) and thus eventual treatment of diabetes¹¹¹.

In addition, VMH dietary obese diabetic rat has been developed by experimental surgical manipulation of genetically normal animals without the reduction in pancreatic beta cell mass resembling type 2 diabetes by combining bilateral electrolyte lesion of VMH and feeding of high fat and high sucrose diets termed as VMH dietary obese rats¹¹². It is characterized by marked obesity, hyperinsulinaemia, hypertriglyceridaemia, insulin resistance, impaired glucose tolerance, moderate to severe fasting hyperglycaemia and defective regulation of insulin secretory response despite extremely high insulin secretory capacity. It is interesting that significant hyperphagia is observed despite increased leptin levels (leptin resistance) in these VMH lesioned rats.

Transgenic and knockout type 2 diabetic models

The nature of marked heterogeneity with multifactorial genetic and environmental background of diabetes poses challenges to identify exact molecular mechanisms involved in treatment of diabetes. Recently, transgenic technique is gaining momentum as it provides excellent opportunity for investigation of role of specific gene products and its mechanisms probably involved in disease conditions under its own physiological (as opposed to *in vitro*) environmental conditions. Transgenic animals are generally helpful in giving insights into gene regulation and development, pathogenesis and finding new targets and the treatment of disease. In general, transgenic animals particularly mice are usually created by transferring and altering the site or level of expression of functional gene (transgene) or by deleting specific endogenous genes (knockout) or placing them under the control of alternate promoter regions¹¹³.

There are some good reviews available in the literatures describing the transgenic/knockout animal models of type 2 diabetes¹¹⁴⁻¹¹⁸. The transgenic and knockout models are developed for studying the role of genes and their effects on peripheral insulin action such as insulin receptor, IRS-1, IRS-2, glucose transporter (GLUT 4), peroxisome proliferator activated receptor- γ (PPAR- γ) and tumour necrosis factor- α (TNF- α) as well as in insulin secretion such as GLUT-2, glucokinase (GK), islet amyloid polypeptide (IAPP) and GLP-1 and in hepatic glucose production (expression of PEPCK) associated with development of type 2 diabetes. Further, combination or double knockout mouse models including defect in insulin action and insulin secretion (*e.g.*, IRS-1^{+/-}/GK^{+/-} double knockout) have been produced which clearly illustrate the mechanisms associated with development of insulin resistance and beta cell dysfunction leading to overt hyperglycaemic state in human type 2 diabetes. These above genetically modified animals exhibit various phenotypic features of type 2 diabetes varying from mild to severe hyperglycaemia, insulin resistance,

hyperinsulinaemia, impaired glucose tolerance and others as explained in detail elsewhere^{6,9,114-118}. Very recently, tissue specific knockout mouse models have been achieved, allowing further insight into the insulin action with respect to particular target tissues (muscle, adipose tissue and liver) associated with insulin resistance and type 2 diabetes^{115,117,118}. The transgenic/knockout animals are currently used mostly for the mechanistic study in diabetes research and not usually recommended for screening programme as they are more complicated and costly.

Conclusion

Many of the animal models described apparently share similar characteristic features of type 2 diabetes and have allowed experimentation that would be impossible in humans. None of the known single species is exactly equivalent to human diabetes, but each model act as essential tool for investigating genetic, endocrine, metabolic, morphologic changes and underlying aetiopathogenic mechanisms that could also operate during the evolution of type 2 diabetes in humans. Hence, care must be taken in interpretation and extrapolation of the results obtained from these animal models to humans. In the screening programme of anti-diabetic compounds, it is particularly important to note that some animal models are better suited to screen particular class of anti-diabetic compounds. Since initial medicinal chemistry campaigns and screening, generally require the testing of many compounds in the industrial research environment, use of smaller animal models such as mice, will reduce the expense of producing test materials while some advanced efficacy studies or toxicological examinations which require invasive procedures and large blood and tissue samples, may be facilitated by using animals with large body size such as rat or other non rodents. Further, the selection of particular animal model is particularly depending on the investigator's choice whether to use inbred or outbred, availability of particular strain, aim of scientific strategy, type of drug being sought, institutional financial and facility resources in the type 2 diabetes research and

pharmaceutical drug discovery and development programme. Though there are some limitations like expensiveness, practical difficulties, extreme care and ethical considerations associated with the use of large/non rodent animal species (*viz.*, pigs, dogs and non human primates), the detailed investigations in these diabetic animal species are urgently required for better understanding of the disease mechanisms in much closely similar human situation as well as for discovering newer targets and drugs for the treatment of type 2 diabetes and its complications.

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